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OFFICE OF
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MEMORANDUM

SUBJECT: 061601. Paraquat Dichloride. Response to Registrant Comments on Acute Dermal Toxicity and Developmental Toxicity Studies and Issues Surrounding the Use of These Studies for Risk Characterization

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Background and Request:

In 1995 and in 1996, Zeneca Ag Products had submitted 5 new acute toxicity studies and two new developmental toxicity studies in hopes of having them included in the Reregistration Eligibility Decision document (RED) for paraquat dichloride. The acute studies were submitted after the Toxicology Chapter had already been completed. Due to strict time constraints, the Data Evaluation Records (DERs) could not be written when the new acute studies were submitted and there was no time to compare the new acute studies with the older acute studies that were already summarized in the Toxicology Chapter of the draft RED document.

The older acute toxicity studies had lower Toxicity Category values. Therefore, in light of public safety, the older acute studies were retained in the draft RED document. The two new developmental toxicity studies were submitted in a rebuttal package to the Toxicology Chapter of the draft RED document. These studies had higher no-observable effect levels (NOEL's) than the older developmental studies. The Registrant wanted all the new acute and developmental studies to supersede the older studies.

In response to the Registrant's rebuttal package, the Toxicology Branch (TB-I) reviewed both of the new developmental toxicity studies and all of the new acute toxicity studies. At that time, TB-I was able to compare the new acute toxicity studies with the old acute toxicity studies and determined that with the exception of the acute dermal study, all of the new acute studies could supersede the old acute studies. For the acute dermal study, TB-I needed further discussion from the Registrant before considering whether or not the new study can supersede the old study. For the developmental toxicity studies, in order to select the most appropriate studies for risk characterization, TB-I needed the Registrant to submit litter data for the old studies so that the developmental NOEL's from these studies could be reassessed using the litter data in addition to the fetal data that was already available in the old studies. In addition, TB-I also needed to reassess the maternal toxicity NOEL for each of the old studies, particularly re-reviewing the maternal body weight data from the mouse study, from which the NOEL for maternal toxicity had been estimated. For the rat developmental toxicity studies, the Registrant has proposed (since both studies use the same strain of rat) using an overall NOEL for maternal toxicity and for developmental toxicity (instead of submitting litter data) that is based on a comparison of the dose spreads for both the old study and the new study and selecting the higher NOEL of the two studies that is below the lowest-observable effect level (LOEL) for both studies. TB-I has received the requested information and has been asked to comment on the Registrant's response.

Toxicology Branch Response:

TB-I has reviewed the Registrant's response to the Agency's questions concerning the acute dermal toxicity study and the developmental studies in the rat and the mouse.

For the rat developmental toxicity studies, TB-I has no objection to the Registrant's request of setting the overall maternal and developmental NOEL's for the rat at 3 mg/kg/day, based on combining the results from the old and new studies. The LOEL's for maternal and developmental toxicity in the rat will therefore be set at 5 mg/kg/day.

For the mouse developmental toxicity studies, after re-examining the maternal bodyweight data and additional data available in our files and after examining the litter data for developmental effects, TB-I is willing to raise the maternal and developmental NOEL's to 5 mg/kg/day and the LOEL's to 10 mg/kg/day. For risk characterization purposes, TB-I will still use the old study with the lower NOEL's because the new study was conducted with a different strain of mouse which may not be as sensitive as the older strain.

For the acute dermal study, after much deliberation over the studies in question, TB-I has decided to accept the new rat acute dermal study as the study that would most likely represent the acute hazard to humans via the dermal route. There are too many confounding factors in the rabbit studies to consider them to be sufficiently reliable for extrapolation to humans. In addition, dermal absorption in humans is very low (0.3%). Therefore, the Toxicity Category for paraquat should be changed from I to III.

Detailed Review

Rat Developmental Toxicity Studies

The Registrant generally accepted the Agency reviews of both the old and new rat developmental toxicity studies. Both studies were conducted with the same strain of rat (Alpk:AP$_f$SD) and both were conducted in the same laboratory, although separated by 14 years. In the old study (MRID 00113714), the animals were tested with 0, 1, 5 or 10 mg/kg/day, expressed as paraquat cation on gestation days 6 through 15 (day when sperm detected was designated as day 0) in a volume of 1 ml/100 g bodyweight. In the new study (MRID 43964701), the animals were tested with 0, 1, 3 or 8 mg/kg/day, expressed as paraquat cation on gestation days 7 through 16 (day when sperm detected was designated as day 1) in a volume of 1 ml/100 g bodyweight.

In TB-I's review of the old rat study, the NOEL for maternal toxicity was set at 1 mg/kg/day and the LOEL was set at 5 mg/kg/day based on deaths (2 dams), clinical signs of toxicity and decreased body weight gain at 5 mg/kg/day and these findings plus respiratory distress and histopathological findings in the lungs (edema in the alveoli and polymorph infiltration) and in the kidneys (degenerative changes in the proximal tubules) of the nonsurvivors. The NOEL for developmental toxicity was also set at 1 mg/kg/day and the LOEL was set at 5 mg/kg/day based on delayed ossification of the forelimb and hindlimb digits in both the mid- and high dose groups.

In TB-1's review of the new rat study, the NOEL's for both maternal and developmental toxicity were set at 8 mg/kg/day, the highest dose tested. No effects were observed at any dose level.

The Registrant proposes using an overall NOEL for maternal toxicity and an overall NOEL for developmental toxicity in the rat of 3 mg/kg/day from the new study. This dose level is below the lowest LOEL for the two studies and is a dose level at which no maternal or developmental toxicity is observed. TB-I has no objections to this proposal, particularly after examining one discrepancy between the Registrant's assessment and TB-I's assessment for maternal toxicity. In its original review, TB-I had stated that there were two deaths in the mid-dose group. After examining the individual animal data, this is true, however, the deaths did not appear to be due to paraquat poisoning. Both appeared to be related to dosing errors. Therefore, without the deaths at 5 mg/kg/day, TB-I is more comfortable with the overall maternal NOEL being set at 3 mg/kg/day for both studies. For developmental toxicity, the NOEL for the old study is based on delayed ossification of the forelimb and hindlimb digits. In the new study, this effect was examined more carefully in the manus and pes assessments and no effects were observed at any dose level up to 8 mg/kg/day. Therefore, TB-I has no objection to setting the overall NOEL for developmental toxicity in the rat at 3 mg/kg/day.

Mouse Developmental Toxicity Studies:

The Registrant does not believe that the NOEL's for maternal and developmental toxicity selected by the Agency for the old mouse study are biologically significant (MRID 00096338). In the old study, paraquat dichloride was administered by gavage in 0.5% aqueous Tween to groups of SPF Alderley Park mice at dose levels of 0, 1, 5 or 10 mg/kg/day expressed as paraquat cation from gestation day 6 through 15. The test solutions were administered in a volume of 0.1 ml/10 g of body weight.

The NOEL for maternal toxicity was set at 1 mg/kg/day and the LOEL was set at 5 mg/kg/day based on a reduction in body weight gain in the mid-dose group (14% decrease, $P<0.05$) and the high-dose group (11% decrease, not statistically significant). The NOEL for developmental toxicity was also set at 1 mg/kg/day and the LOEL was set at 5 mg/kg/day based on an increase in partially ossified 4th sternebrae in the mid- and high-dose groups. It was reported that 85% of the control group fetuses had good ossification of the sternebrae, compared with 76% and 74% of the mid-dose and high-dose fetuses, respectively.

Maternal Toxicity

The Registrant states that "the 1978 study shows a decrease in body weight gain at 5 and 10 mg/kg/day. However, there is no dose response. This is contrary to the known steep dose response characteristic of paraquat." Therefore, they believe that this is not a toxicologically significant effect. The following tables summarize the mean bodyweight and bodyweight gain data for

the dams during gestation. These data were either taken directly from the study or were calculated from the mean bodyweight data provided in the study.

Mean Maternal Bodyweights During Gestation (g)				
Dose (mg/kg/day)	Gestation Day			
	0	6	15	18
0	31.0	34.7	53.0	62.6
1	31.1	34.2	51.1	61.6
5	32.6	36.3	50.5	59.8
10	32.1	35.0	50.9	60.3

[a]Percent of control value

Mean Maternal Body Weight Gain (g)

Interval	Dose in mg/kg/day (# of Dams)			
	0 (20)	1 (22)	5 (28)	10 (23)
Pretreatment: Days 0 - 6	3.7	3.1 (84)[a]	3.7 (100)	2.9 (78)
Treatment: Days 6 - 15	18.3	16.9 (92)	14.2 (78)	15.9 (87)
Posttreatment: Days 15 - 18	9.6	10.5 (109)	9.3 (97)	9.4 (98)
Days 6 - 18	27.9	27.4 (98)	23.5 (84)	25.3 (91)
Entire Gestation Period: Days 0 - 18	31.6	30.5 (97)	27.1 (86)*	28.2 (89)
Approximate Corrected Bodyweight Gain[b]	15.7	14.4 (92)	12.6 (80)	12.9 (82)

[a]Percent of the control value.
[b]Corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus mean litter weight.
* $p < 0.05$

In a range-finding study for the developmental toxicity study, 7 non-pregnant SPF Alderley Park mice/dose were dosed for 10 days by gavage at the following dose levels: 5, 10, 20 or 40 mg/kg paraquat ion. The animals were observed for an additional

10 days. All the animals died at 40 mg/kg/day during the latter stages of the dosing period or up to 4 days after cessation of dosing. One animal died at 10 mg/kg/day on day 14. There were no deaths at 20 mg/kg/day. Bodyweights were recorded on day 1 and 5 days after cessation of dosing. The following table summarizes the mean body weights.

Mean Bodyweights on Day 1 and Day 15			
Dose Level (mg/kg/day)	Day 1	Day 15	Bodyweight Gain (Days 1-15) (g)
5	33.0	33.7	0.7
10	32.9	32.0	-0.9
20	36.9	33.7	-3.2
40	All animals died		

In two range-finding studies conducted with the same strain of mouse in 1979 (MRID's 00087921 and 00087922) in preparation for the oncogenicity study in the mouse, groups of 20 male and female mice were fed paraquat cation in the diet at 0, 3.75, 7.5, 11.2, 15, 18.7 and 30 mg/kg/day and at 0, 1.9, 3.7 and 7.5 mg/kg/day, respectively. For comparison purposes, only the results from the females are mentioned in this memorandum. Fourteen of 20 females died at the 30 mg/kg/day dose level during test weeks 2-4. With the exception of the highest dose level, there were no statistically significant differences in bodyweight gains when compared to the control groups. At 18.7 mg/kg/day, pulmonary congestion, edema and collapse and kidney congestion were observed at study termination in some animals.

Based on all of the data provided above and on the data provided below in the developmental toxicity section, TB-I is willing to raise the NOEL for maternal toxicity to 5 mg/kg/day and the LOEL to 10 mg/kg/day. This decision is based mainly on the one death at 10 mg/kg/day in the range-finding study and on the statistically significant increased incidence of litters with delayed ossification of the 4th sternebrae at 10 mg/kg/day which may be indicative of maternal toxicity in addition to the decreases in bodyweight gain at both 5 and 10 mg/kg/day. Since the decreases in bodyweight gain at 5 mg/kg/day were not supported by any other data, this decrease is not considered to be a biologically significant effect. Effects were observed in the other oral studies with this strain of mouse at 18.7 mg/kg/day and above. The new developmental toxicity study in the mouse was conducted with a different strain (Crl:CD1(ICR)BR) and thus may not be as sensitive as this particular strain.

Developmental Toxicity

The Registrant states that although the historical control data were limited, it indicates that "there was an unusually high incidence of '4th sternebra - partially ossified' across all groups (*including control*) with *no* apparent dose response to paraquat." The Registrant also states that the effect on ossification was only statistically significant at 10 mg/kg/day. In addition, they consider "that if the ossification pattern of the 4th sternebra was affected by paraquat, a continuum of changes to the 4th sternebra would be expected. This is not seen, as evidenced by the lack of increase in '4th sternebra - not ossified.'" The following table summarizes the fetal and litter data for partially ossified 4th sternebrae and for 4th sternebra - not ossified.

Fetal and Litter Data for Non-Ossified and Partially Ossified 4th Sternebrae: # Fetuses Affected/# Litters Affected

Dose (mg/kg/day)	0	1	5	10
# Fetuses/# Litters Examined	120/20	130/22	156/28	137/23
Non-ossified sternebrae	1/1	0/0	0/0	0/0
Partially ossified sternebrae	18/8[a]	17/12	37/14	37/18[b]
% affected (fetuses/litters)	15.0/40.0	13.1/54.5	23.7/50.0	26.3[c]/78.3

[a]Statistically significant increasing trend ($p = 0.02$): Cochran-Armitage test
[b]Statistically significant increase in # litters affected ($p = 0.012$): Fisher's Exact test
[c]Statistically significant at 0.05% probability

After examining the fetal and litter data above, TB-I has decided to increase the NOEL for developmental toxicity to 5 mg/kg/day and the LOEL to 10 mg/kg/day. Although there was a lack of increase in 4th sternebra: not ossified, since there were statistically significant increases in both the fetal and litter data, TB-I cannot disregard the data at the high dose.

Acute Dermal Studies:

The old acute dermal study is characterized as Toxicity Category I (conducted with the rabbit) and the new acute dermal study (conducted with the rat) is characterized as Toxicity Category III. The Registrant states that paraquat is poorly absorbed through the skin in the rat and in man and that a

Toxicity Category rating of I is too severe. They state that the old study indicates that there was possible oral ingestion, particularly because one survivor of the 250 mg/kg treatment group had a very rough, irregular tongue consistent with oral ingestion of paraquat in the rabbit. Paraquat is known to be highly toxic to rabbits when ingested. The acute oral LD_{50} ranges between 20 and 100 mg/kg. The Registrant also mentioned another acute dermal study published in 1972 which stated that "animals (rabbits) wearing restraining collars to prevent oral ingestion showed no mortality and caused only minor reversible systemic symptoms of intoxication at a rate of 480 mg/kg when application was made to an uncovered area."..."When free grooming was allowed, residual skin contamination caused severe tongue ulceration [severe glossitis] and inability or unwillingness to eat." The LD_{50} is considered to be greater than 480 mg/kg. According to a 1966 copy of the study in the Agency files (MRID 00046103), in another experiment in the study, a single application of 240 mg cation/kg was applied and covered with a completed occluded dressing. No attempt was made to decontaminate the skin after 24 hours. The animals all died by 72 hours. There was no evidence of glossitis. This study showed that the tongue lesions are most likely due to oral ingestion and not caused by dermal absorption.

A comparison was made from the results of the old acute dermal study and the old dermal irritation study (both of which were conducted with male New Zealand White rabbits (abraded and intact) and used the same paraquat product for the same exposure period) further confounds the issues. The acute dermal LD_{50} was reported to be 174 mg/kg. Using a few assumptions, it was calculated that in the old dermal irritation study, the minimum amount that the rabbits received was 215 mg/kg. One animal died. This indicates that the LD_{50} is at least greater than 215 mg/kg, which is not far off from 174 mg/kg, but is at the bottom end of toxicity category II. Since the dermal irritation study report stated that "most of the dose was observed to have dried on the gauze patch and not on the skin", the actual acute dermal LD_{50} may actually be less. However, the same may have happened in the acute dermal study as well (although not stated). Using the same assumptions for the new dermal irritation study, there were no deaths in 3 rabbits which were exposed to 162, 195 or 213 mg/kg, respectively. However, this exposure was only for 4 hours instead of 24 hours.

TB-I searched the Agency files for other acute dermal studies conducted with paraquat on rabbits. Several studies surfaced, some of which were conducted with a 29.1% concentrate (MRID Nos. 00153415, 00114455, 00090967, 00046103, 00033206). The data from these studies generally places paraquat in either Toxicity Category I or II, more often II. However, as the Registrant states, there appears to be a problem with oral ingestion by the rabbits, even in those studies where collars

were placed on the animals and some covering was placed over the site throughout the observation period. The LD_{50}'s in the studies that used methods that were supposed to prevent oral ingestion were higher, but the LD_{50}'s were still generally in Toxicity Category II. Glossitis of the tongue was present in many of the animals, particularly those in the higher dose levels. Intraperitoneal injection of paraquat in rabbits (LD_{50} approximately 18 mg ion/kg bodyweight) did not induce glossitis. Glossitis was also not present in one animal and/or a group (not totally clear) that had been dosed with 240 mg/kg and then covered with a totally occlusive dressing. The test site was not decontaminated after 24 hours and the animal(s) died after 72 hours. In this study, however, there was some question about changes in the skin that happen when totally occlusive dressings are used, thus not representing actual exposure conditions in the field. In the same paper, paraquat labelled with C^{14} was applied to the skin as in a normal study. Paraquat was applied to the shaved back of a rabbit and exposed to the air for 24 hours. The rabbit was prevented from licking the site of application (not stated how). After decontamination (washing with warm water and gently dried), it was determined that less than 0.1% of the applied dose remained on the skin. Using these results and the acute oral LD_{50} of rabbits (20-100 mg/kg), in those studies in which the application site was washed, a question arises as to how much of the oral contamination contributes to the acute dermal LD_{50} in the rabbit studies. Obviously, it does contribute some because the rabbits do not eat when glossitis is present and they become anorexic, thus exacerbating any toxicity.

Lastly, it appears that paraquat is probably dermally absorbed at a higher rate in the rabbit than in the rat. Some of the reports stated that dermal absorption of ions is greater in the rabbit than in either rats or humans. One report states that humans have the lowest rate of dermal absorption of ions than several other animals with rabbits having the highest rate. Thus, studies in the rabbits would exaggerate potential toxicity for humans. In TB-I's files, we have a dermal absorption study in humans that states that only 0.230-0.295% of a radioactive dose was absorbed when applied to the hands, legs and forearms of 6 adult male volunteers. In the human study, the exposed areas were not washed for 24 hours and urine was collected for 5 days following dosing.

After a review of all the data summarized above, TB-I has decided to accept the new rat acute dermal study as the study that would most likely represent the acute hazard to humans via the dermal route. There are too many confounding factors in the rabbit studies to consider them to be sufficiently reliable for extrapolation to humans.